30. (NEW) The process as claimed in claim 20, additionally comprising filtration of the thrombin preparation through a membrane with a suitable pore size to remove viruses.

31. (NEW) A thrombin preparation, which is obtainable by the process of claim 20.

32. (NEW) A method of using the thrombin preparation of claim 18 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

33. (NEW) A method of using the thrombin preparation of claim 19 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

34. (NEW) A method of using the thrombin preparation of claim 31 as a hemostatic, a constituent of a hemostatic or as a constituent of tissue glue.

REMARKS

Claims 1-17 have been cancelled. New claims 18-34 have been added and correspond to claims 1-17 as filed. Applicants submit that these amendments conform the claims to United States Patent and Trademark Office practice and, as such, introduce no new matter.

Several paragraphs of the specification have been replaced in order to correct informalities and place trademark names in proper United States Patent and Trademark Office format.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

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GARRETT & DUNNER, L.L.P.

Dated: March 15, 2001

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APPENDIX TO PRELIMINARY AMENDMENT

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 8, lines 6-14, with the following:

Initially the thrombin solution was mixed with 0.6 mol/l sodium sulfate and adsorbed onto a hydrophobic interaction chromatography (HIC) gel (in this case: Phenyl-Sepharose HP, manufacturer: Amersham Pharmacia, Freiburg, Germany) which had previously been equilibrated with buffer A (10 mmol/l Na phosphate pH 6.5) containing 0.6 mol/l sodium sulfate. After washing with buffer A containing 0.6 mol/l sodium sulfate, the bound thrombin was eluted by a gradient with decreasing sodium sulfate content in buffer A. Impurities and thrombin fragments were to a large extent removed in the flow-through or in the wash fractions.

Please replace the paragraph at page 8, lines 15-26, with the following:

The thrombin fraction was loaded without further treatment directly onto a cation exchange column (CEC; in this case: [Fractogel®] FRACTOGEL® EMD SO_3, manufacturer: Merck, Darmstadt, Germany) equilibrated with buffer A, washed with equilibration buffer A and eluted by a gradient from 0 to 1.0 mol/l sodium chloride in buffer A. During the separation, final byproducts and thrombin fragments were removed so that the resulting α-thrombin eluate had a high specific purity of about 3500 IU/mg [[](protein determination by determining the absorption at 280 nm and using the conversion factor of 1.74 for a 0.1% strength solution in accordance with J.W. Fenton,

Thrombins. J Biol Chem 252; 3587-3598 (1977)[[]]). Table 1 shows the results of this thrombin purification and the resulting specific activity.

Please replace the paragraph at page 9, lines 3-14, with the following:

Starting from a thrombin concentrate of moderate or low purity, two chromatography steps were carried out. Initially the thrombin solution was mixed with 0.6 mol/l sodium sulfate and adsorbed onto a hydrophobic interaction chromatography (HIC) gel (in this case: Phenyl-Sepharose HP, manufacturer: Amersham Pharmacia, Freiburg, Germany) which had previously been equilibrated with buffer B (10 mmol/l Na phosphate 0.1% PEG pH 6.5; [[](in this case PEG 6000, but other molecular weight ranges can also be employed)[]]) containing 0.6 mol/l sodium sulfate. After washing with buffer B containing 0.6 mol/l sodium sulfate, the bound thrombin was eluted by a gradient with decreasing sodium sulfate content in buffer B. Impurities and thrombin fragments were to a large extent removed in the flow-through or in the wash fractions.

Please replace the paragraph at page 9, lines 15-19, and page 10, lines 1-3, with the following:

The thrombin fraction was loaded without further treatment directly onto a cation exchange column (CEC; in this case: [Fractogel®] FRACTOGEL® EMD SO_3, manufacturer: Merck, Darmstadt, Germany) equilibrated with buffer C (10 mmol/Na phosphate, 166 mmol/l L-arginine pH 6.5), washed with equilibration buffer C and eluted by a gradient from 0 to 1.0 mol/l sodium chloride in buffer C. During the separation,

eluate had a high specific purity of about 3300 IU/mg [[](cf. Table 2).

Please replace the paragraph at page 10, lines 15-16 and page 11, lines 1-7, with the following:

Starting from a thrombin eluate purified as in Examples 1 to 3 and after hydrophobic interaction chromatography and cation exchange chromatography, a filtration was carried out on a membrane with a small pore size (e.g. [Planova] PLANOVA™ 15 nm). Even small viruses such as parvoviruses can be effectively removed with this membrane. It was found that on use of the purified thrombin as starting material, very good yields in terms of thrombin activity and protein were obtained, with a good filtration rate (see Table 3). This process is therefore suitable for producing a thrombin concentrate with high virus reduction factors.

Please delete the paragraph at page 16, line 1 as follows:

[AVENTIS BEHRING GMBH 2000/A002-A1]

Please replace the paragraph at page 16, line 2, with the following:

[Patent claims for the USA] We claim: